Title: Increasing the maturity of compost used affects the soil chemical properties and the stability of microbial activity along a Mediterranean post-fire chronosequence

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Abstract

Compost addition has been largely employed to improve chemical properties and microbial activities of several disturbed soils. However, few attempts have assessed the adequacy of compost quality considering the level of ecosystem recovery after frequent wildfires in combination with droughts. We investigated the suitability of the addition of 3 ages of compost (i.e. 3 weeks, 3 months and 9 months) crossing with 3 times since fire (i.e. 1, 5 and 18 years of recovery) to increase the soil organic and inorganic resources in frequently burned soils. We hypothesised that resource depending on quality (i.e. maturity) should improve microbial activity and its resistance and resilience against a drastic drought and could have some implication for SOM mineralisation. Our results showed that the more mature compost, richer in organic matter, increases TOC, total N, PO_4^{3-}-P concentrations and pH but regardless the time since fire. Microbial activity weakly responded to this soil resource improvement whereas it was strongly depressed 5 years after the last fire. Mature compost resulted in a loss of resistance and resilience of the microbial activity in comparison with control soils depending on the time since fire, indicating that exogenous resource as compost affects microbial stability. The cumulative C-mineralisation clearly indicated that the loss of microbial activity and stability against drought with the more mature compost would result in an improvement of soil C-accumulation especially 5 years after the last fire.

Keywords: Organic amendment; microbial respiration; soil; resistance; resilience; C-accumulation.

Introduction

Since the 1970s, Mediterranean basin has been marked by socio-economic mutations and an increase in extreme weather events, such as heat waves and droughts (Gibelin and Déqué, 2003), favouring the occurrence of extended wildfires and frequency (Pausas and Fernández-Muñoz, 2011). Frequent fires decrease soil organic matter and nutrients (Guénon et al., 2011, 2013a) and lasting affect the microbial functional resistance (ability to withstand climate stress) and the resilience (i.e. time necessary to return to the pre-stress level) against climatic stress (Guénon and Gros, 2013b). Drying-rewetting cycles killing sensitive microbial populations induce a pulse in microbial CO_2 emission and then, can reduce C-mineralisation which has some importance for soil C-sequestration (Fierer and Schimel, 2003). At ecosystem scale, frequent wildfires exacerbated by drought events in next decades could impair the recovery of ecological functions supported by soil microbes and thus, some ecosystem services such as carbon sequestration.

Amendment with organic wastes is frequently used to help in the re-establishment of abiotic and biotic soil properties after fires (Guerrero et al., 2001; Kowaljow and Mazzarino, 2007; Larchevêque et al., 2005; Ros et al., 2003; Turrión et al., 2012; Villar et al., 1998;) and is encouraged to restore degraded soils. Compost amendments can improve soil physical, chemical and biological properties, especially by increasing available nutrients in the organic soil fractions (Larchevêque et al., 2006a). Biosolid composts are rich in humified organic matter and can be used as a slow-release nutrient source (Barker, 1997). They have also a high water retention capacity (Giusquiani et al., 1995) which induces an increase of soil water content (Villar et al., 1998). These modifications can positively affect plant cover through an improvement of plant nutrition and growth (Guerrero et al., 2001; Larchevêque et al., 2005, 2006b), and contribute to reduce erosion (Guerrero et al., 2000). Compost addition is frequently referring to improve soil microbial biomass and activities (Borken et al., 2002;

Kowaljow and Mazzarino, 2007) but most studies were carried out either under controlled conditions with short incubation experiments or either in the field with only descriptive effects. We propose in this study to combine both the field and the laboratory experiments to test our hypotheses. Currently, little attention has been paid to the effects of organic amendment directly in the field in interaction with abiotic stress like drying and rewetting events on i) the microbial activity and its capability to resist and recover (Hueso et al., 2012) ii) the mineralisation of soil organic matter (Turrión et al., 2012) and iii) the potential implications for C-accumulation (Adani et al., 2009).

Compost addition, by improving nutrient availability, pH or the carbon content and its availability, can favour resistance and resilience (i.e. stability) of microbial functions (Hueso et al., 2011). The level of soil enrichment depends on the quality of the compost used (Guerrero et al., 2001). Kowaljow and Mazzarino (2007) showed that biosolid compost richer in carbon and nitrogen content than municipal compost better improves chemical and microbial properties 12 months after *in situ* amendments. Conversely, an addition of fresh organic matter in a Mediterranean area, lesser improved the soil chemical and microbial properties than a composted organic matter less rich in total carbon and nitrogen (Ros et al., 2003). Therefore, the use of compost on burned soils requires to test interaction effects between the chemical properties of the compost used and transfer to soil to assess the resistance and resilience of microbial activity against a drastic stress (i.e. drying and rewetting event) and study the potential implications for soil C-accumulation.

In this study, we examined the potential effect of compost amendments on microbial activity and its stability (i.e. resistance and resilience) against an experimental drought, and consequently, C-accumulation in a Mediterranean post-fire chronosequence. We previously detected a threshold in SOM quality and quantity between 4 and 17 years of time since fire that controls the recovery of microbial activities (Guénon et al., 2011). Moreover, we also tested the role of C and N availability in controlled conditions on the stability of microbial functions against droughts (Guénon and Gros, 2013b). Thus, in the current study, we hypothesised that the chemical quality of composts (i.e. maturity depending on time of composting) would control microbial activity, depending on the time since fire, its resistance and resilience that feedback the whole process of C-accumulation. More precisely, we expected that young compost, richer in labile organic compounds and nutrients, would favour the stability of recently burned soil (i.e. lower level of resources) by increasing microbial activities. These effects should be attenuated along the post-fire chronosequence (i.e. recovery of resource availability) and would increase soil C-accumulation. The specific objectives were thus, to assess the effects of 3 compost ages (i.e. 3 weeks, 3 months and 9 months) added to 3 frequently burned soils differing by time since fire (i.e. 1, 5 and 18 years of recovery) on i) soil resource content (total organic C, total N, total P, NO_3^-–N, NH_4^+–N, PO_4^{3-}–P), ii) resistance and resilience of microbial basal respiration to an experimental drying and rewetting event (D/Rw), iii) relationships between soil chemical properties and basal respiration and its stability to D/Rw and iv) cumulative C-mineralisation.

2. Material and Methods

2.1. Study area

The study was conducted in part of the Maures moutain range (Var, southern France, 43°20’ N and 6°37’ E). The region is characterised by a typical Mediterranean climate with 920 mm of mean annual rainfall and 14°C of mean annual temperature (1962-2003). The study area (90 km^2) presents a range of altitude from 100 to 400 m above sea level. The mother rock is a gneiss migmatitic (crystalline siliceous rock). Soils along the post-fire chronosequence have a sandy loam texture and are classified as Dystric Leptosol (IUSS

Working Group WRB, 2006). The study area is characterised by heterogeneous mosaic of Mediterranean forest ecosystems generated by various wildfire frequencies (Schaffhauser et al., 2012). Plant communities that recover in the first years following fire are dominated by herbaceous (e.g. *Bituminaria bituminosa* L., and Lotus species) and young fast growing woody species (e.g. *Cistus monspeliensis* L., *Calycotome spinosa* L., *Erica arborea* L.) and also tall *Quercus suber* L. that survived to fires. In the late successional stage (i.e. with no fire for at least 59 years), highly covered forests are dominated by a tree canopy of *Quercus suber* L., *Quercus ilex* L. and *Pinus pinaster* Aiton subsp. pinaster on maquis.

2.2. Experimental design and soil sampling

The burned surfaces were mapped using a series of aerial pictures spanning a 57-year period from 1950 to 2007 and public fire database (Prométhée, 2007). This map was interpreted in order to select study sites according to the number of fires since 1950 and to the time since fire. Nine sites (1000 m^2) were selected because they were similar in terms of number of fires (i.e. 4 fires). This frequency corresponds to a critical fire regime for the northern Mediterranean Basin (Guénon et al., 2011). Wildfire regime also differed by time since the last fire constituting an atypical Mediterranean post fire chronosequence rarely studied. Sites were categorized as follows:

- 3 independent unburned sites for 1 year (referred as “1y” in Table and or figures). These sites just begun their recovery in term of plant communities (see above). Total elements are close to older sites (Table 1) due to the supply of burned plant material that may counterbalance the combustion of organic matter (Certini, 2005). It was however expected both low resource quality (i.e. heterocyclic compounds) and nutrient availability (González-Pérez et al., 2004).

- 3 independent unburned sites for 5 years (referred as “5y” in Table and or figures). These sites did not recover for plant community structure (80-90% covered by C*istus monspeliencis* L.) and soil chemical and microbial properties were strongly affected (Guénon et al., 2011, 2013b)
- 3 independent unburned sites for 18 years (referred as “18y” in Table and or figures). These sites completely recovered in term of plant communities’ assemblage (Schaffhauser et al., 2012), total C and N content but did not recover for its quality, nutrient availability or all microbial functions (Guénon et al., 2011, 2013a).

All these sites presented substantial level of total element (Table 1) but modulated by the quality of resource that control the microbial activities (Guénon et al., 2011, 2013a). We brought different compost qualities expecting that each quality should be adapted to different burned situations. Wildfires occurred in summer under harsh drought and strong wind and were considered as intense, and also because burned surface had a similar level of post-fire mortality for *Quercus suber* L. (Schaffhauser et al., 2012). The main chemical and microbiological characteristics of the burned soils are given in Table 1.

The compost was produced by a local company (Biotechna, Ensuès-La-Redonne, France). It was made with municipal sewage sludge mixed with pin barks and green wastes (1/3 v:v). After being composted for 20 to 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, the mixture was sieved (<40-mm mesh) to remove the large bark pieces and stored as windrows. The windrows were mixed several times over the next 8 months to promote organic matter maturation. Three composts maturities were selected according to the time of composting i.e. 3 weeks (3wC), 3 months (3mC) and 9 months (9mC) and thus, to their differences in physico-chemical and microbiological characteristics (Table 2). In august 2008, composts were surface-applied (i.e. mulch) at a rate of 70 Mg (dry equivalent matter) ha^{-1} on 3 independent plots (1 m^2 each) delimited on the 9 burned sites. A fourth adjacent plot was delimited and non-amended to serve as control (NC) for the compost treatment. Each plot was fixed to soil with wooden boards and metal hooks to prevent the loss of compost by torrential rain. Moreover, a metal grid was fixed to wooden boards to prevent disruption of composted-soil by wild boars.

For each plot, after removing the litter and compost layer from the soil surface, the A horizon (0 to 5 cm depth) was sampled in January 2009 and again in June 2009 (5 and 10 months after compost application, respectively) from half of the surface of the 1 m^2 plots (i.e. 0.5 m^2 for each sampling time). Soils were immediately sieved (2 mm mesh size) and kept at 4°C before chemical and microbiological analyses were conducted.

2.3. Soil chemical characteristics

Soil total organic carbon (TOC) and total nitrogen (TN) content was measured on air-dried samples using a C/N elemental analyzer (Flash EA 1112 series ThermoScientific). The total phosphorus (TP) content was determined according to Sparrow et al. (1990) after an extraction (1N H_2SO_4) of ignited samples (540°C, 16h). The same extraction of un-ignited samples was used to determine inorganic P. The filtered extracts were analysed colorimetrically for orthophosphates as described in Guénon et al. (2011b). Inorganic-N forms (NH_4^+–N and NO_3^-–N) were extracted (10 g dry weight equivalent of moist soil, 100 ml KCl 1M, shaking 1 hour) and colorimetrically analysed by respectively nitroprusside-salicylate method and nitrosalicylic acid method as described in Guénon et al. (2011). Soil pH was assessed by a soil-water suspension (1/2.5) two hours after shaking.

2.4. Microbial basal respiration and biomass

Basal respiration (BR) was measured to assess the ecophysiological state of soil microbial communities. Ten g (dry weight equivalent) of fresh soil were placed in 117 ml glass jars and then pre-incubated for 4 days at 22°C to allow microbial respiration to restart. The glass jars were then closed with hermetic rubber septa, and incubated for 4 hours (22°C). After incubation, 1 ml of air was sampled in the head space with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO_2 production. The gas chromatograph was equipped with a thermal conductivity detector and a packed column (Porapack). The carrier gas helium flow was regulated at 60 ml h^{-1}. Ambient CO_2 concentrations were subtracted from sampled CO_2 concentrations and resulting values were adjusted at 22°C according to Ideal Gas Laws using a $Q_{10} = 2$. BR was expressed in µg CO_2-C (g dry soil)$^{-1}$ h^{-1}.

Active microbial biomass (MB) was estimated using substrate induced respiration (SIR) rates (Anderson and Domsch, 1978). Ten grams (dry weight equivalent) of fresh sub-samples were placed in 117 ml glass jars and amended with powdered glucose (1000 µg C g^{-1} soil) that maximises the respiration rate in our soils (data not shown). Immediately after glucose amendment, samples were exactly incubated during 1.5 hours, then air flushed and the glass jars were closed and incubated during 1.5 hours. One ml of air was sampled in the head space with a syringe and injected into a gas chromatograph to analyse CO_2 production (see above). SIR rates were converted into MB using equations given by Beare et al. (1990). MB was expressed in µg Cmic (g dry soil)$^{-1}$. Metabolic quotient (qCO_2) was obtained by dividing the basal respiration to the microbial biomass (BR/MB).

2.5. Measurement of soil microbial resistance and resilience

For each of the 36 soil samples (i.e. 4 compost treatments x 3 times since fire x 3 repeated plots), 2 equal sub-samples of 10 g (dry weight equivalent) of fresh soil were placed in 117 ml glass jars. Seven days after an incubation stage in optimal condition of temperature (25°C) and humidity (60% of the water holding capacity: WHC), the first lot of sub-samples received a drying and rewetting event (D/Rw) while the second lot of sub-samples was maintained in optimal conditions throughout the experiment (control soils ‘C’). The D/Rw event was composed of 2 phases: i) a drying period of 72 hours at 50 °C allowing to reach a final water content less than 2 % of WHC, ii) a rewetting period with a fast return of moisture content equivalent to 60 % WHC at 25 °C.

Soil microbial respiration was measured, as described above, 10 hours after the rewetting phase, to assess resistance, and after 34, 58, 82, 164 and 236 hours to assess resilience. The moisture content was kept constant throughout the experiment. Resistance and resilience of microbial activity against D/Rw event were defined as the capacity to maintain their level of activity near their respective control soils (‘C’). Percentage of control soils permitted both to interpret the effect of D/Rw event and to compare effect of the time since fire in combination with addition of composts. Resistance (RT) and resilience (RL) were calculated as follows:

$$\text{RT and RL (\%)} = [D / C] \times 100$$

where D is the measured value of soil microbial basal respiration submitted to the D/Rw event. C is the relative measure of activity in unstressed soils (control soils).

2.6. Effect of drying/rewetting event on cumulative CO_2 respiration

To express the potential consequence of a combining effect of drying and rewetting event with an input of compost on the loss of soil organic carbon, we calculated the cumulative microbial respiration throughout the experiment (see above) expressed in mg of CO_2-C per gram of total organic carbon and by day (mg CO_2-C g^{-1} OC d^{-1}).

2.7. Statistical analyses

Two-way analyses of variance (ANOVA) were used to determine the effects of time since fire (Tsf) x Age of compost (AC) on soil chemical properties (total organic C, total N, total P, NH_4^+-N, NO_3^--N, PO_4^{3}-P and water pH) and also, on the soil microbial basal respiration before the application of a drastic drying and rewetting event. These analyses were performed both 5 and 10 months after compost addition. Since no effect was found after 5 months, we only present results after 10 months. When a significant interaction was found, we separately analysed the effects of AC for each Tsf by one-way ANOVA followed by least significance tests (LSD, $P<0.05$) to analyse in detail the variations between each modality of compost treatment. In contrast, if no significant interaction was found, but main effects were significant, data were analysed with one-way ANOVA to detect differences only for the factor AC, because time since fire alone is not debated in this study. Since two-way ANOVA only revealed a single significant interaction (Tsf x AC) for chemical analyses (i.e. total phosphorus), we only showed results of one-way ANOVA for compost effects in table 3.

We used two-way repeated measures of ANOVA (rmANOVA) to test the interaction effects of Tsf and AC within time after rewetting, on the soil microbial respiration expressed as the percentage of control soils (i.e. to assess resistance and resilience) and the cumulative respiration expressed by carbon unit. Since we found significant interactions between Tsf, AC and time after rewetting, we separately analysed the effect AC for each Tsf and for each time after rewetting by one-way ANOVAs followed by LSD tests ($P<0.05$). Data were $\log_{10}$ transformed when necessary to meet the assumption of normality and homogeneity of variances. These analyses were performed on Statistica 6.0.

Stepwise multiple regression analyses were used to determine which combinations of variables mostly explained variation in soil microbial activity before and after stress and its resistance and resilience. Only variables that remained significant at $P<0.05$ were retained.

Explanatory variables for basal respiration and its resistance and resilience were the soils chemical analyses (TOC, TN, total P, NH_4^+–N, NO_3^-–N, PO_4^{3-}–P, pH) and the microbial biomass. These analyses were performed on SPSS 12.0.

3. Results

3.1. Effect of the age of compost and time since fire on soil physico-chemical and microbial properties

Total phosphorus content was significantly changed depending on both, the time since fire and the age of composts ($F=2.99$, $p<0.05$ for “Tsf x AC” interaction). Indeed, total P enrichment was lower for the 5 years of Tsf plots with 3 month-aged compost (3mC) and intermediate with 9 month-aged compost (9mC). Conversely, the 3 week-aged compost (3wC) increased twice the total P content in comparison with non-composted soil (Figure 1). For the 1 and 18 years of Tsf plots, the total P content doubled with the addition of the 3 composts ($P<0.05$; Figure 1). In contrast, any significant interaction between compost maturity and time since fire was found on TOC, total N, inorganic P and soil pH. Compost addition significantly increased total content of organic carbon (main effect, $F=5.7$, $p<0.01$), nitrogen ($F=8.6$, $p<0.001$), inorganic phosphorus ($F=18.5$, $p<0.001$) and soil pH ($F=7.7$, $p<0.001$) especially with the most aged composts (Table 3). Enrichment in total elements was stronger for N and P than C as revealed by a significant decrease in C/N and C/P *ratios* (Table 3).

Addition of different age of composts significantly changed the soil microbial respiration depending on the time since fire ($F=2.98$, $P<0.05$ for “AC x Tsf” interaction, Fig. 5). Indeed, both the 3mC and 9mC decreased the microbial activity for the 5 years of Tsf plots and composts did not presented significant effect for the 1 and 18 years of Tsf plots (Fig. 2, Histograms). Microbial biomass slightly increased with the age of composts but this effect was not significant (ANOVA test, P>0.05; Table 3). Metabolic quotient was not affected by the compost addition (P>0.05).

3.2. Immediate effect of a drastic D/Rw event on soil microbial respiration: the Resistance

The applied drying and rewetting event (D/Rw) significantly changed the soil microbial basal respiration (BR) and its stability (BR%) expressed as % of control soil (unstressed) depending on the time since fire (Tsf), the age of compost (AC) and the time after the rewetting (P<0.05 for "Tsf x AC x Time after rewetting" interaction, Fig. 5).

For the 1 year of Tsf plots, all the composts slightly but significantly increased the BR by 1 unit (Figure 2a). This resulted in an increase in BR% with the 3wC (190 %) in comparison with NC, 3mC and 9mC that reached only 160 % (averaging) (Figure 3a). For the 5 years of Tsf plots, only the 3 week-aged compost (3wC) increased the BR reaching more than 8 µg CO_2-C g^{-1} dry soil h^{-1} in comparison with NC, 3mC and 9mC that reached only 6 µg CO_2-C g^{-1} dry soil h^{-1} averaging (Figure 2b). This resulted in an increase in BR% for the 3 composts (230 % averaging) in comparison with NC that reached only 130 % (Figure 3b). For the 18 years of Tsf plots, we observed an initial increase in BR depending on the age of compost. Indeed, the 9 month-aged compost (9mC) presented a higher activity (12.8 µg CO_2-C g^{-1} dry soil h^{-1}) in comparison with non-composted (NC) soils that reached 8 µg CO_2-C g^{-1} dry soil h^{-1} (Figure 2c). This resulted in a loss of resistance (RT) corresponding to a relative increase in BR% higher than 270 % of control soils (i.e. unstressed soils) in comparison with the 3mC and NC that reached only 210 % (averaging, LSD test, P<0.05) (Figure 3c).

3.3. Temporal effects of a drastic D/Rw event on soil microbial respiration: the Resilience

Between 10 and 58 hours after rewetting, microbial basal respiration (BR) decreased quickly, the lower slope for the 1 year of Tsf plots and the higher slope for the 18 years of Tsf (Fig. 2).

For both 1 and 18 year of Tsf plots and from 58 hours after rewetting, the BR in composted soils stabilised (i.e. plateau) reaching the same level as NC soils (Figures 2a and 2c). This resulted in a significant decrease in BR% under the 3mC and 9mC treatments for the 1 year of Tsf plots (Figure 3a) and a decrease in BR% under the 3mC in comparison with NC soils for the 18 years of Tsf plots (Figure 3c). An atypical effect was found for the 5 years of Tsf plots in NC soils that maintained BR up to 34 hours after rewetting. The BR finally decreased for the 3mC and 9mC treatments below the NC soil level until the end of the experiment (Fig. 2b). Thirty four hours after rewetting the BR% was significantly higher for the 3wC treatment and finally BR% decreased under the NC soil for the 3 composts treatments until the end of the experiment (Fig. 3b).

3.4. Relationships between physico-chemical properties and microbial biomass in explaining the BR and BR%

We used stepwise multiple regressions to determine, within each time since fire, which combinations of variables explained most of the variations in basal respiration across treatments before and after a drastic drying/rewetting event (D/Rw) and its stability (Table 4). Before D/Rw, total organic carbon content explained positively the variation in basal respiration (BR) for the 1 and 18 year of Tsf plots, but no relationships were found for 5 years of Tsf plots (Table 4).

For the 1 year of Tsf plots, total organic carbon positively explained the response of microbial activity (BR) after the drying and rewetting event. From 34 hours after rewetting, soil pH and phosphate content significantly improved the models, the later finally replaced by nitrate content from 164 hours until the end of the experiment (Table 4). The stability of microbial activity (BR%) was only explained from 236 hours after rewetting by total P and nitrate content (Table 4).

For the 5 years of Tsf plots, total organic carbon content was significantly and positively related to the BR 10 hours after rewetting soil (Table 4). From 164 hours after rewetting until the end of the experiment, the BR was better explained by soil pH in a negative way. Stability of basal respiration (BR %) was negatively explained by the inorganic phosphorus both at 164 and 236 hours after rewetting (Table 4).

For the 18 years of Tsf plots, total organic carbon content strongly related to the response of basal respiration (BR) after D/Rw event (Table 4). Moreover, total nitrogen improved the model at each step of resilience and nitrate content improved the model both at 58 and 236 hours after rewetting (Table 4). Stability of basal respiration (BR%) was negatively explained by the available nitrate both at 58 and 82 hours after rewetting and by inorganic phosphorus 236 hours after rewetting (Table 4).

3.5. Cumulative respiration expressed by organic carbon unit

Cumulative respiration (CR) expressed by organic carbon unit (mg CO_2-C g^{-1} OC d^{-1}) significantly changed depending on the time since fire, the age of compost and the time after rewetting ($F=2.30$, $P<0.001$ for "Tsf x AC x Time after rewetting" interaction, Fig. 5). We observed 2 phases separated by a shift in relationships between 58 and 82 hours after rewetting (Figure 4). The first period (i.e. between 10 and 58 hours after rewetting) corresponded to the maximum slope while the second period (i.e. between 58 and 236 hours), corresponded to a slow-down and stabilisation in the cumulative respiration.

For the 1 year of Tsf plots, the CR was significantly higher in NC soils than the 3mC and 9mC treatments. The CR under 3wC was not significantly different to NC soils but was different to 3mC and 9mC (LSD test, $P<0.05$, Figure 4a).

For the 5 years of Tsf plots and between 34 hours after rewetting until the end of experiment, the CR was significantly higher in NC soils than both 3mC and 9mC and to a lesser extent than 3wC treatment (Figure 4b).

For the 18 years of Tsf plots and from 58 hours after rewetting, the CR was significantly higher in non-composted soils (NC) than soils that received the 9 month-aged compost (9mC) (Figure 4c).

4. Discussion

In Mediterranean ecosystems, wildfire is the main disturbance that affects soil organic matter content and nutrient availability (e.g. N, P) which in turn controls the recovery of plants and microbial functions (Carreira and Niell, 1992; Hart et al., 2005). Organic amendments as compost can be used to speed up the natural recovery of soil properties.

In this study, ten months after compost addition was the time necessary to find significant changes in soil chemical properties (Table 3). Contrariwise to our hypotheses, only phosphorus content changed depending on both time since fire and compost maturity. Indeed, the older the compost was, the higher it increased the total organic carbon and nitrogen (Table 3) that could be imputed from a higher content of organic matter (Table 2) (Kowaljow and Mazzarino, 2007). Otherwise, changes in soil chemical properties followed the maturity of compost (Table 2) but regardless to time since fire thus contradicting our initial hypothesis. The quantity of compost that we brought to our burned soils has probably hidden the effect of the time since fire (Table 1) which controls the soil resource content (Guénon et al., 2013a). Conversely, nitrate and ammonia content did not change with compost addition while it has been reported as a major risk for eutrophication (Guo and Li, 2012). We suggest, in context of low nitrogen availability in burned Mediterranean soils (Guénon et al., 2013a) that plant uptake and microbial immobilisation could regulate inorganic nitrogen content (Guerrero et al., 2001) despite significant differences between the current composts used (Table 2).

Despite the few interactions on soil chemical properties (see above and Table 3), microbial activity as basal respiration, its stability (i.e. resistance and resilience against a drastic drought) and cumulative C-mineralisation strongly responded to both time since fire and compost amendment (i.e. interactions highly significant, Figure 2, 3, 4 and 5). Addition of the older compost (i.e. 9 month-aged) richer in organic matter did not improve the microbial basal respiration after 1 and 18 years of time since fire. This indicates, contrariwise to other studies (Borken et al., 2002; Saison et al., 2006), that a strong resource input brought to soil did not necessarily change the microbial physiological status while we previously demonstrated that this activity was C and N limited in these burned soils (Guénon and Gros, 2013b). However, we detected a change in C:N:P stoichiometry (Griffiths et al., 2012) with compost addition that could explain this lack of increasing microbial respiration. Indeed, the C/N and C/P decreased with compost addition (Table 3) and could have limited C-availability for microbial respiration, but need further investigations. Even more, five years after the last fire, mature composts decreased microbial basal respiration (Figure 2b) that we cannot explain by variations in soil chemical properties (Table 4). Borken et al. (2002) reported a similar decrease in O-horizon with mature compost addition and attributed this effect to the low microbial activity in mature compost. In our burned soils, this horizon does not exist but we suggest that compost directly in contact with A-horizon could generate the same decrease in microbial activity due to a more stable organic matter. This indicates that soil microbial communities in this fire regime would be not-adapted to this resource quality. Otherwise, since microbial biomass did not change, we suggest that addition of composts could have changed microbial communities (Saison et al., 2006) for the benefit of microbial population with lower C-rate. However, these last authors demonstrated that compost-borne micro-organisms do not persist or are not active in soil where environmental conditions are very different than in compost. We thus suggest that the addition of compost, by profoundly modifying soil chemical conditions of these burned soils might equilibrate the relationships between soil native microbes and compost-borne micro-organisms resulting in a strong competition for resource and lower C-rate. Also, we cannot rule out a possible inhibitory effect of element trace metals (ETM) lixiviated from such mature compost (Larchevêque et al., 2010). Indeed, some ETM as copper, zinc and also chrome were higher in both aged composts (i.e. 3mC and 9mC, see Table 2) even if they are largely under the legal French limit (e.g. Larchvêque et al., 2010). These higher concentrations could explain a depressed microbial activity but it is not clear in this study why other post-fire steps (i.e. 1 and 18 years after fire) were not affected. Transfer in soil of ETM and bioavailability needs to be verified.

Rewetting dry soils induced a CO_2 pulse, referred as a “Birch effect” (Birch, 1958), which is a consistent response with several other studies (e.g. Fierer and Schimel, 2003). This phenomenon consists in an increase of microbial respiration probably caused by the mineralisation of dead microbes by those which survived and also, by an increase in available carbon, previously protected against microbial attack, released after aggregate slaking (Cosentino *et al.*, 2006). In this study, the effect of drying and rewetting was modulated by the time since fire as we hypothesised (Fig. 5). Amendment of the more mature compost, improving soil organic matter content, increased this pulse in microbial respiration in comparison with non-composted soils and for all the times since fire. The stepwise multiple regressions (Table 4) confirmed that changes in organic resources are the primary driver of the intensity of this ‘Birch’ effect. Moreover, we assume that this phenomenon could be partially imputed to a supplementary loss in microbial biomass which had been increased by the more mature composts (Table 3). Our results suggest that this organic resource by increasing biomass may have resulted in a loss of stability, which could be explained by selective effect of less resistant microbial communities (Hueso et al., 2011). However, it has been suggested that larger C and N content would contribute to a significant microbial stability (Wardle, 1998). Our results show that resource content cannot alone explain microbial stability as demonstrated by Guénon and Gros (2013b) i) regarding non-composted soils that increased the percentage of control soils against drought, ii) with an increase in time since fire (i.e. recovery of soil resource) and iii) with compost addition that clearly affected the stability (Fig. 5). Indeed, these last authors previously demonstrated that an experimental enrichment of C and N in these burned soils, increasing microbial size and resource availability, did not change the stability of microbial basal respiration against drought. In the current study, stepwise multiple regressions showed that the strong enrichment in inorganic phosphorus could explain the low ability of microbial communities to resist and recover from extreme drought for the three times since fire. This could indicate that the role of resource availability on the stability of microbial activity could depend on the life strategy of soil microbial communities (i.e. energy allocation) rather than its content, which permits microbial growth. Also, the high increase in organic resources could have changed the soil microbial communities in these frequently burned soils, probably less adapted to drought and thus, inducing supplementary death of microbes (Hueso et al., 2011). This hypothesis should be verified by assessing potential changes in microbial community composition or diversity.

Finally, five years of time since fire seems to be a critical stage of the post-fire chronosequence (see above) that should not receive mature compost since microbial activity was affected before and after drying and rewetting event and also presented a better resistance to drought without compost addition (Fig. 3b and 5). Additionally, the resilience of microbial activity in this fire regime was also affected by all the composts falling down below 50 % of activity of control soils. These results confirm that the soil microbial communities of this fire regime are adapted to extreme drought.

Addition of organic-C using compost is one practice that can improve carbon sequestration in soil (Adani et al., 2009). Our results indicated that the more mature composts decreased mineralisation of organic carbon 5 years after the last fire, revealing potential soil C-accumulation for this regime and also, this effect was amplified by extreme drought event (Figure 2b). However, in order to better evaluate the consequence of compost addition combined with hydric stress on soil C-dynamic, the cumulative microbial respiration expressed by carbon unit was calculated (Figure 4). Our results indicated that the combination of C-enrichment and drought significantly decreased carbon mineralisation that may confirm a potential implication for soil C-sequestration over time (Fierer and Schimel, 2003). According to our results, we suggest that addition of mature compost in Mediterranean ecosystems submitted to frequent wildfires and drought should increase C-sequestration over time. This process would be the lowest in the very initial step of the post-fire chronosequence with the more mature compost and would increase between five and eighteen years after fire.

5. Conclusions

Addition of compost to frequently-burned-Mediterranean soils increased soil resource content after 10 months depending on the age of compost but regardless on the time since fire. Secondly, both the resistance and resilience of basal respiration to extreme drought decreased with compost addition, especially 5 years after fire with all composts (Fig. 5), despite the soil enrichment in organic and inorganic resources. Thirdly, variation in total organic content was the main driver of microbial activity, while variation in nutrient content explained microbial stability. According to our hypotheses, younger compost were better adapted to recently burned soils, while older burned plots also better responded to this compost quality (older compost affected all properties, Fig. 5). We detected one combination of fire and compost that never hampered microbial properties: the 3 week aged-compost added to 18 years of burned soils (Fig. 5). However, we showed a decrease in microbial C-mineralisation increasing with compost maturity (Fig. 5), that would result in a greater C-accumulation in soil, but could nevertheless impair ecosystems services such as plant productivity and the recovery of Mediterranean ecosystems.

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Table 1: Chemical and microbial properties of burned soils at the beginning of the experiment

Time since fire (years)	1y	5y	18y
Chemical properties			
TOC ($g.kg^{-1}$)	44.9 ±9.4	43.3 ±6.5	55.9 ±14.6
TN ($g.kg^{-1}$)	2.9 ±0.5	2.3 ±0.3	3.1 ±1.3
TP ($g.kg^{-1}$)	0.49 ±0.05	0.42 ±0.11	0.48 ±0.06
C/N	15.5 ±1.3	19.3 ±1.8	18.5 ±3.2
C/P	95 ±31	108 ±35	116 ±21
NH_4^+–N ($mg.kg^{-1}$)	21.3 ±1.8	22.7 ±2.8	57.2 ±22.2
NO_3^-–N ($mg.kg^{-1}$)	18.3 ±1.1	9.5 ±1.3	18.4 ±4.7
PO_4^{3-}–P ($g.kg^{-1}$)	0.39 ±0.08	0.29 ±0.11	0.25 ±0.09
Soil pH (in water)	6.4 ±0.1	6.8 ±0.1	6.4 ±0.1
Microbial properties			
Basal respiration (µg CO_2-C (g dry soil)$^{-1}$ h^{-1})	3.4 ±0.7	3.4 ±0.5	4.6 ±0.3
Microbial biomass (µg Cmic (g dry soil)$^{-1}$)	1.2 ±0.2	1.7 ±0.3	2.2 ±0.4
qCO_2 (µg CO_2-C (µg C_{mic})$^{-1}$ h^{-1})	2.92 ±0.62	2.06 ±0.41	2.22 ±0.44

Values are means ± standard deviation

Table 2: Physico-chemical and microbial properties of the three composts used

Properties	Methods	3wC	3mC	9mC
Total elements (g kg^{-1})				
Total organic carbon	NF EN 13039	174	260	268
Total nitrogen	NF EN 13654-2	14.1	20.0	20.0
Total phosphorus	NF EN 13650	7.1	7.0	7.1
Organic matter (%)	NFU 44-160	58.7	57.5	67.6
NO_3^-–N	Mulvaney (1996)	0.002	0.059	0.112
NH_4^+–N	Keeney & Nelson (1982)	2.87	2.42	1.89
Potassium	NF EN 13650	4.3	6.6	6.6
Calcium	NF EN 13650	36.4	68.8	64.3
Magnesium	NF EN 13650	2.1	3.2	3.2
pH	Soil/water (1/2.5)	8.5	8.3	7.9
Copper (mg kg^{-1})	NF EN ISO 11466	134.4	173.8	176.8
Zinc	NF EN ISO 11466	268.0	331.8	331.5
Cadmium	NF EN ISO 11466	0.8	0.8	0.8
Chrome	NF EN ISO 11466	16.3	20.6	20.4
Mercury	NF EN ISO 11466	0.3	0.5	0.4
Nickel	NF EN ISO 11466	11.5	12.7	12.6
Lead	NF EN ISO 11466	30.4	47.3	38.1
Organic matter fractions and indexes:				
Soluble fraction (SOL)*	Van Soest & Wine (1963)	47.6	39.5	41.9
Hemicellulose (HEM)*	Van Soest & Wine (1963)	8.1	8.0	6.5
Cellulose (CEL)*	Van Soest & Wine (1963)	19.9	26.0	27.8
Lignine + cutin (LIC)*	Van Soest & Wine (1963)	24.3	26.5	23.8
Crude cellulose*	Weende	36.5	37.2	42.9
Biological stability index (BSI)	Linière & Djakovitch (1993)	0.36	0.53	0.37
(C=C + C=O) / Asym C–H *ratio* (1633/2920 cm^{-1})	Haberhauer *et al.* (1998)	2.9	2.5	3.2

Table 2: Continues

Physical properties :				
Electrical conductivity (mS cm^{-1})	Water extract (1/1.5)	5.95	4.15	2.96
Microbial properties :				
Density of culturable bacteria[a] (Colony-forming unit g^{-1} DM)	Albrecht *et al.* (2010)	$1.75\ 10^6$	$1.78\ 10^6$	$1.72\ 10^7$
Density of culturable fungi[b] (Colony-forming unit g^{-1} DM)	Albrecht *et al.* (2010)	$2.23\ 10^4$	$5.9\ 10^4$	$1.19\ 10^6$

* % of OM ; [a] culture on yeast peptone glucose agar. [b] culture in melting malt extract agar . Abbreviations: 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost.

Table 3: Effect of the age of compost on soil chemical and microbial properties 10 months after amendment

	ANOVA test		Compost treatments			
	F	p	NC	3wC	3mC	9mC
Total Organic C ($g\ kg^{-1}$)	5.7	<0.01	48 ±4 a	72 ±6 ab	81 ±7 bc	98 ±14 c
Total N ($g\ kg^{-1}$)	8.6	<0.001	2.8 ±0.3 a	5.0 ±0.5 b	5.8 ±0.6 bc	6.7 ± 0.8 c
C/N	10.4	<0.001	18 ±1 b	15 ±0.5 a	14 ±0.5 a	14 ±0.5 a
C/P	4.4	<0.05	106 ±9 c	66 ±6 a	81 ±6 ab	94 ±12 bc
NH_4^+-N ($mg\ kg^{-1}$)	ns	ns	18 ±1 a	20 ±1 a	20 ±1 a	19 ±1 a
NO_3^--N ($mg\ kg^{-1}$)	ns	ns	33 ±3 a	42 ±4 a	38 ±4 a	39 ±5 a
PO_4^{3-}-P ($g\ kg^{-1}$)	18.5	<0.001	0.3±0.1 a	1.0±0.1 b	1.1±0.1 b	1.1±0.1 b
Soil pH (in water)	7.7	<0.001	6.7±0.1 a	7.0±0.1 b	7.1±0.1 bc	7.3±0.1 c
Microbial biomass	ns	ns	1.72 ±0.14 a	1.78 ±0.11 a	1.83 ±0.17 a	2.06 ±0.25 a
qCO2	ns	ns	1.97 ±0.17 a	2.09 ±0.18 a	1.99 ±0.23 a	1.81 ±0.11 a

Abbreviations: NC: non-composted soils; 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost. Microbial units are given in Table 1. Mean values (±standard deviation) followed by the same letters were not significantly different at P<0.05 (LSD test). Values for each compost modality were given whatever the wildfire regime (interaction not significant. P>0.05). ns: not significant.

Table 4: Relationships between soil properties and microbial activity (BR) and its stability (BR %) against a drastic drying/rewetting event (D/Rw)

	Before D/Rw		Resistance 10h after Rw		Resilience 34h after Rw		58h after Rw		82h after Rw		164h after Rw		236h after Rw	
Time since fire	Model	R^2	Model	R^2	Model	R^2	Model	R^2	Model	R^2	Model	R^2	Model	R^2
1 year														
BR	TOC(+); pH(-)	0.91***	TOC(+)	0.64**	TOC(+); pH(-); PO_4^{3-}(+)	0.90***	TOC(+); pH(-); PO_4^{3-}(+)	0.94***	TOC(+); pH(-)	0.73***	TOC(+); pH(-); NO_3^-(+)	0.93***	TOC(+); pH(-); NO_3^-(+)	0.91***
BR %	n.p.		n.s.		n.s.		n.s.		n.s.		n.s.		TP(-); NO_3^-(+)	0.68**
5 years														
BR	n.s.		TOC(+)	0.62**	TOC(+); pH(-)	0.74**	n.s.		n.s.		pH(-); TOC(+)	0.63*	pH(-)	0.48*
BR %	n.p.		n.s.		n.s.		n.s.		n.s.		PO_4^{3-}(-)	0.43*	PO_4^{3-}(-)	0.69***
18 years														
BR	TOC(+); TN (-)	0.75***	TOC(+)	0.72***	TOC(+); TN (-)	0.84***	TOC(+); TN (-); NO_3^-(-)	0.91***	TOC(+); TN (-)	0.81***	TOC(+); TN (-)	0.83***	TOC(+); TN (-); NO_3^-(-)	0.93***
BR %	n.p.		n.s.		n.s.		NO_3^-(-)	0.45*	NO_3^-(-)	0.41*	n.s.		PO_4^{3-}(-)	0.63**

Abbreviations: BR: basal respiration; BR %: basal respiration after D/Rw expressed as percentage of control soils (unstressed); D/Rw: drying and rewetting. The models showed the combination of chemical variables and microbial biomass that maximises R^2 and only the significant variable at P<0.05 were included n=12 for each time since fire before rewetting the dry soil and at resistance and resilience. * P>0.05. ** P<0.01; ***P<0.001 and n.s. not significant. n.p. not performed.

Figure captions:

Figure 1: Effect of the time since fire and the age of compost on total phosphorus content in soils 10 months after amendment. Means with the same letters were not significantly different (LSD test. P<0.05). NC: non-composted soils; 3wC: 3 week-aged compost: 3mC; 3 month-aged compost; 9mC: 9 month-aged compost.

Figure 2: Effect of the age of compost depending on time since fire on microbial basal respiration before (histogram) and after drying/rewetting experiment (on the basis of 2 way-repeated measures ANOVA). Histograms: means with the same letters were not significantly different (LSD test, P<0.05). * indicates significant effect of compost treatment for a given time after rewetting (LSD test, P<0.05). For clarity, replicates of each treatment were averaged. NC: non-composted soils; 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost.

Figure 3: Effect of the age of compost depending on time since fire on resistance (RT) and resilience (RL) of soil microbial activity against a drastic drying and rewetting event (on the basis of 2 way-repeated measures ANOVA). * indicates significant effect of compost treatment for a given time after rewetting (LSD test, P<0.05). For clarity, replicates of each treatment were averaged. NC: non-composted soils; 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost.

Figure 4: Effect of the age of compost depending on time since fire on cumulative microbial respiration expressed by carbon unit after rewetting dry soils (on the basis of 2 way-repeated measures ANOVA). * indicates significant effect of compost treatment for a given time after rewetting (LSD test, P<0.05). For clarity, replicates of each treatment were averaged. NC:

non-composted soils; 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost.

Figure 5: Schematic synthesis of the compost effects depending on time since fire on microbial activity (BR) before and after a drastic drying and rewetting event (D/Rw), the stability (BR%), as resistance and resilience and cumulative C-mineralisation (CR) . Middle size circles indicate the level in non-composted (NC) soils. The same size for compost amendment indicates no significant change in property while smaller circles indicate a decrease and the bigger indicate an increase in property. NC: non-composted soils; 3wC: 3 week-aged compost; 3mC: 3 month-aged compost; 9mC: 9 month-aged compost.

Codes, as follows 1, 5 and 18 refer to 1, 5 and 18 years after the last fire, respectively.

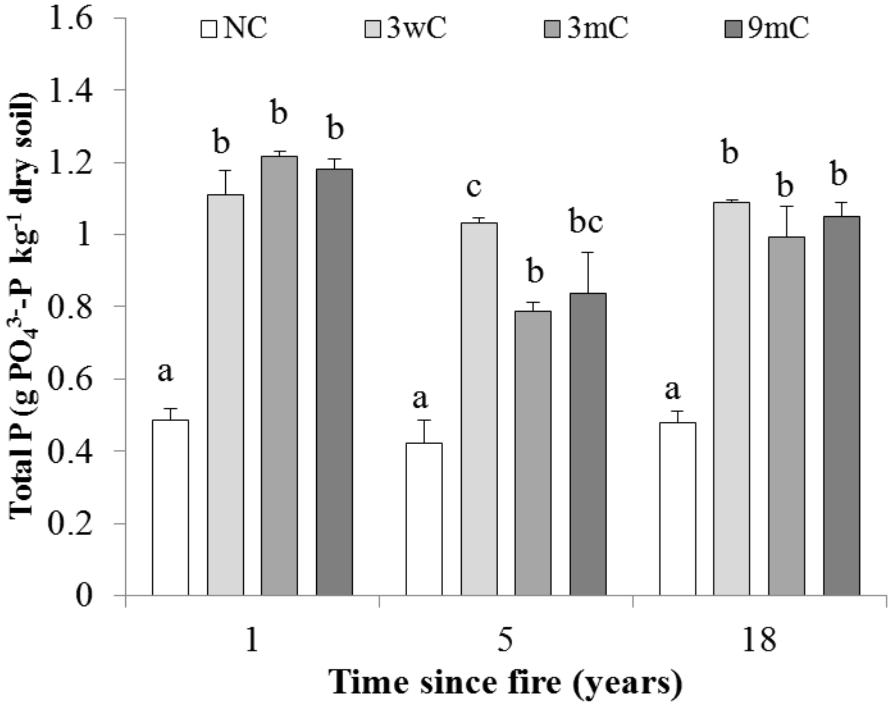
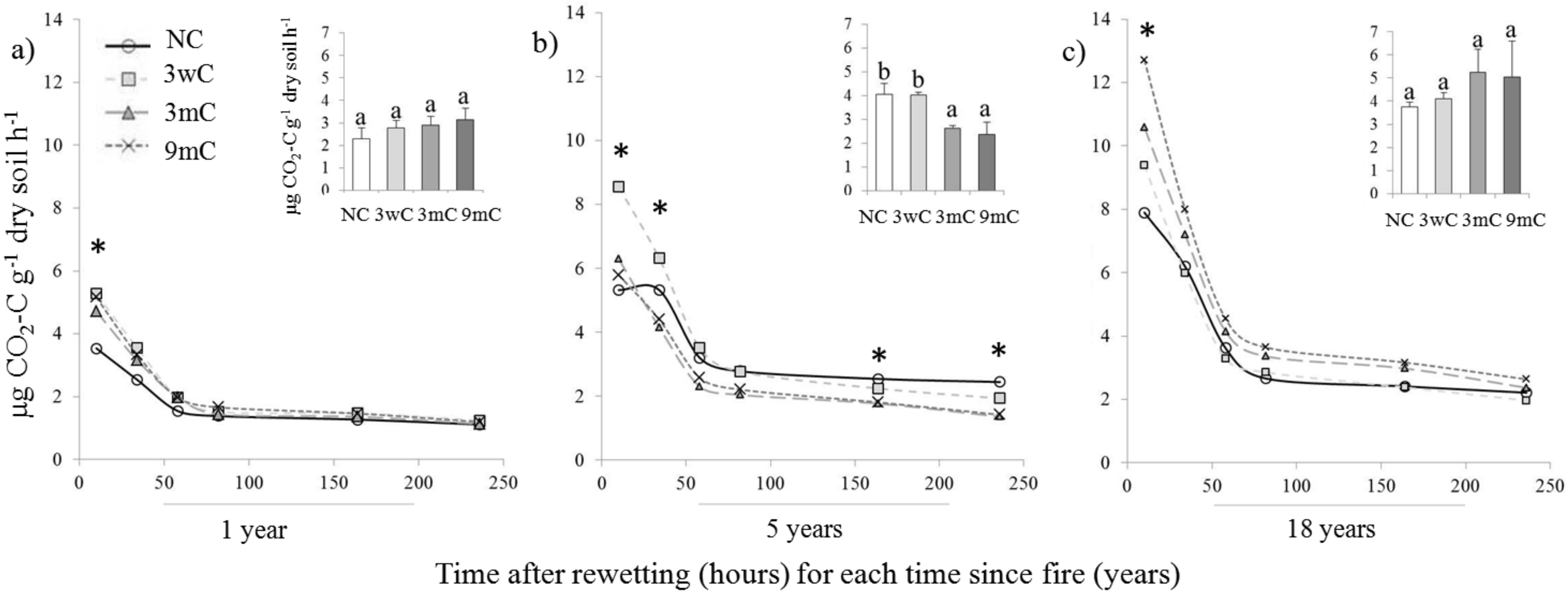
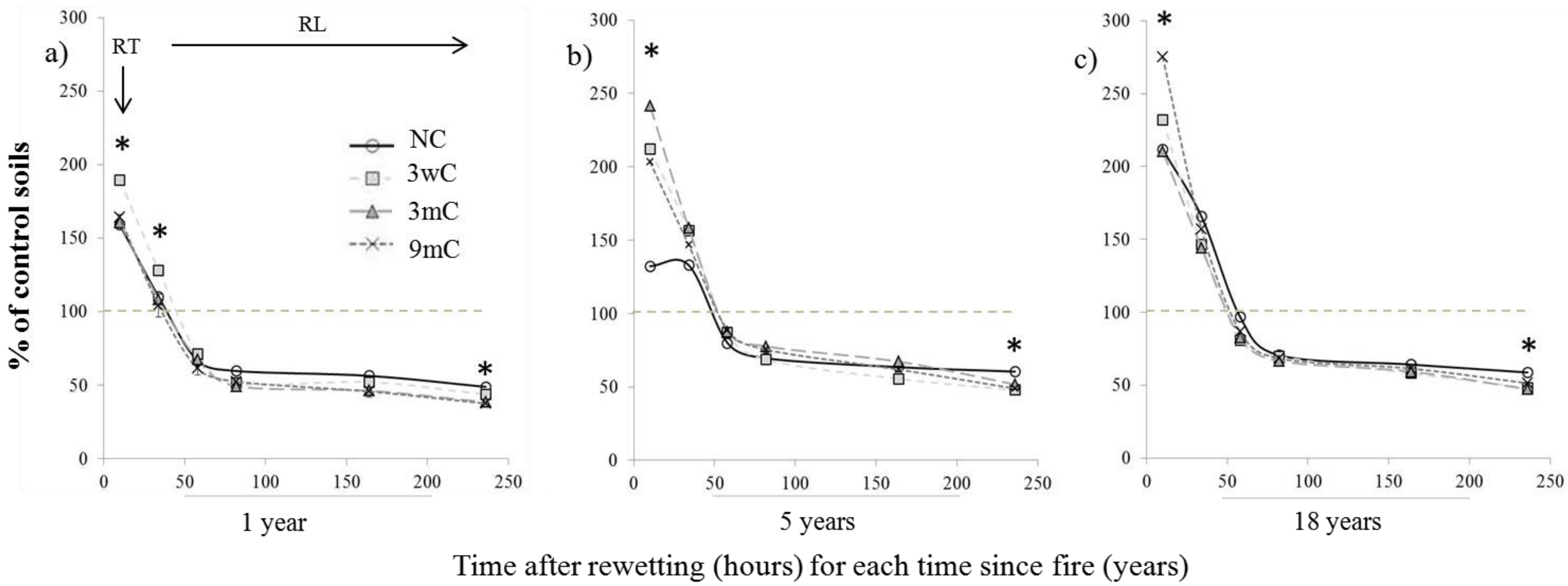

NC
3wC
3mC
9mC
Total P (g PO_4^{3-}-P kg^{-1} dry soil)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
a
c
b
bc
a
b
b
b
1
5
18
Time since fire (years)

Fig. 1



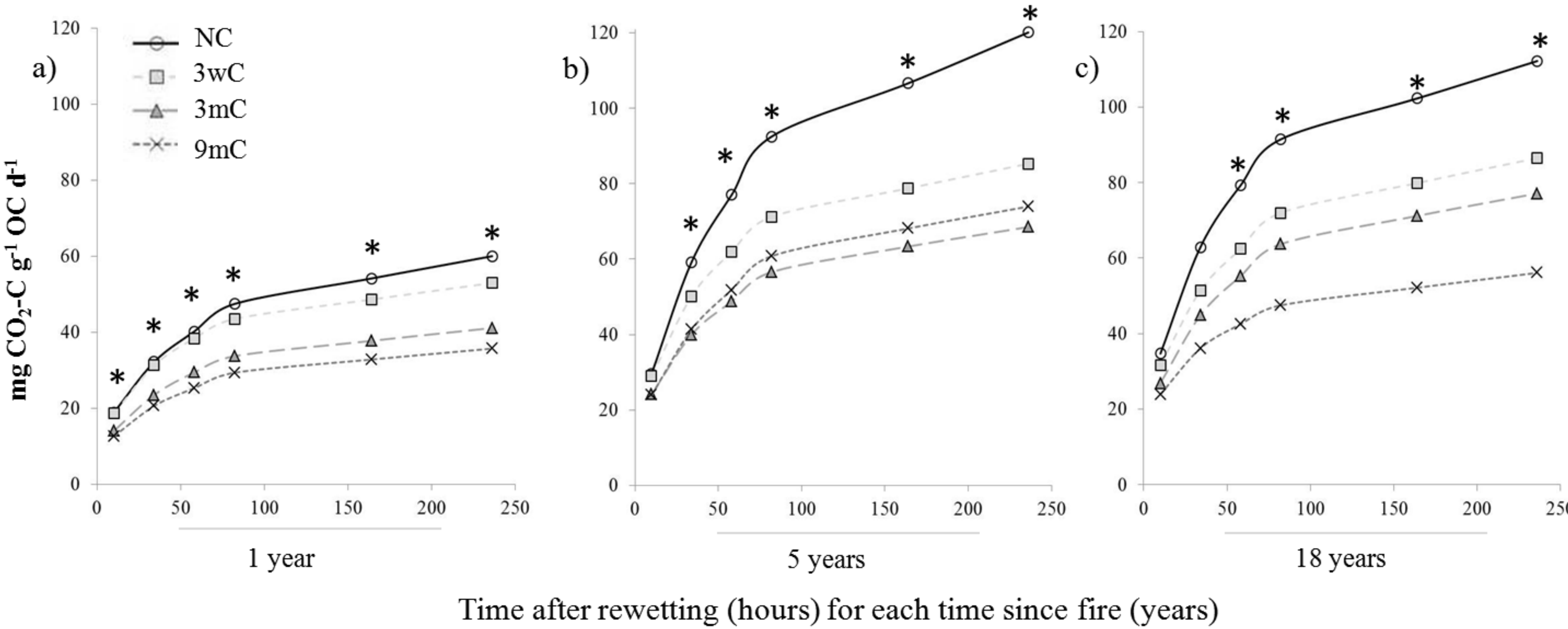
a)
b)
c)
NC
3wC
3mC
9mC
μg CO2-C g-1 dry soil h-1
NC 3wC 3mC 9mC
1 year
5 years
18 years
Time after rewetting (hours) for each time since fire (years)

Fig. 2



RL
a)
RT
NC
3wC
3mC
9mC
% of control soils
1 year
b)
5 years
c)
18 years
Time after rewetting (hours) for each time since fire (years)

Fig. 3



a)
b)
c)
NC
3wC
3mC
9mC
mg CO_2-C g^{-1} OC d^{-1}
0
20
40
60
80
100
120
0
50
100
150
200
250
1 year
5 years
18 years
Time after rewetting (hours) for each time since fire (years)

Fig. 4

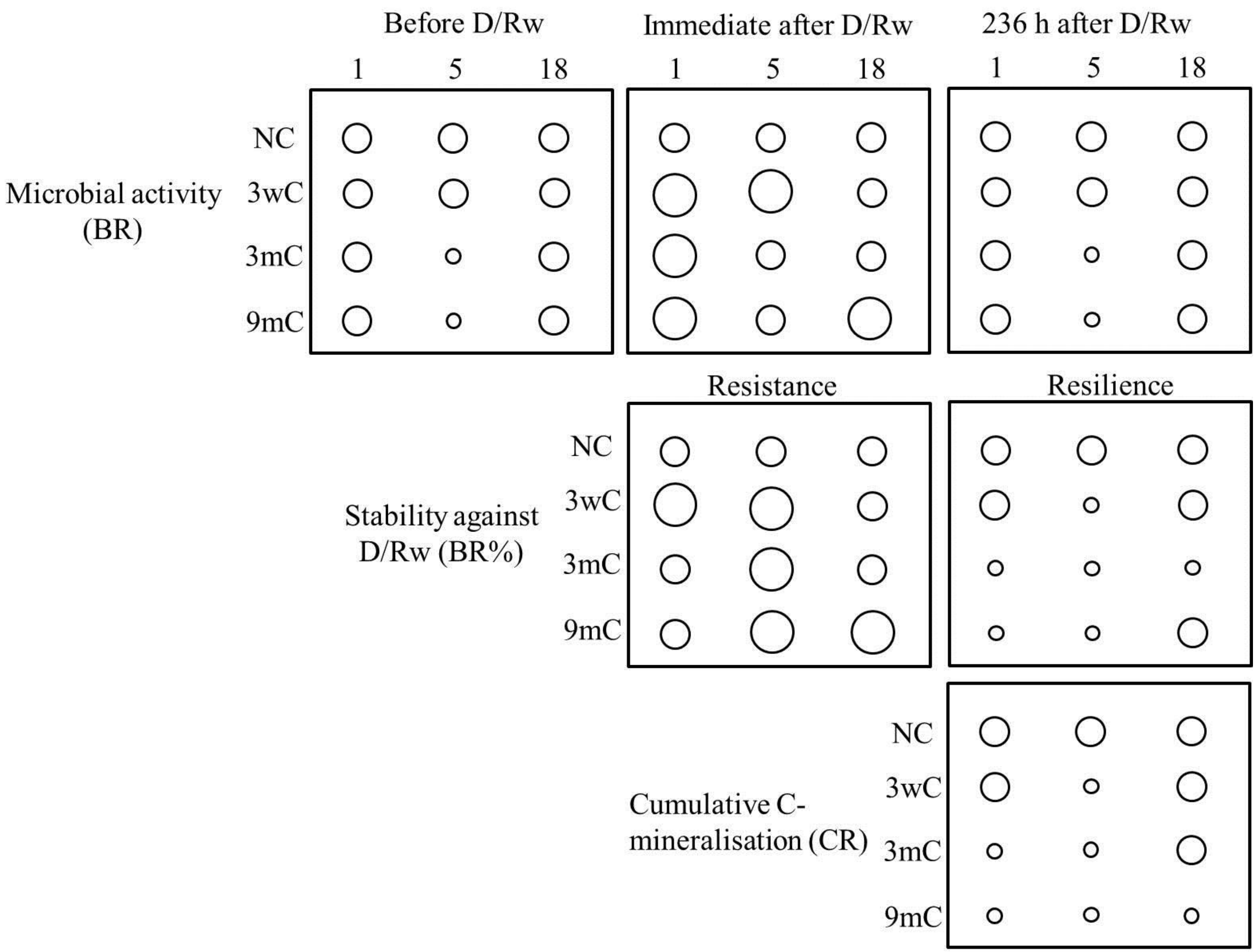

Before D/Rw
Immediate after D/Rw
236 h after D/Rw
1
5
18
Microbial activity (BR)
NC
3wC
3mC
9mC
Resistance
Resilience
Stability against D/Rw (BR%)
Cumulative C-mineralisation (CR)

Fig. 5